

THAT WHICH IS CLAIMED:

1. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- 5 (a) a nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 15, or 17;  
(b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 16, or 18;  
(c) a nucleotide sequence characterized by at least 88% sequence identity to the nucleotide sequence set forth in (a);  
10 (d) a nucleotide sequence encoding a protein comprising an amino acid sequence characterized by at least 85% sequence identity to the amino acid sequence set forth in (b);  
(e) an antisense nucleotide sequence corresponding to the nucleotide sequence of any one of (a) to (d); and  
15 (f) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of any one of (a) to (e).

2. The nucleic acid according to claim 1, wherein the nucleotide sequence is optimized for expression in a plant.

20 3. An expression cassette comprising a nucleic acid according to claim 1, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a microorganism or in a plant cell.

25 4. An isolated pesticidal polypeptide selected from group consisting of:  
(a) a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 2, 4, 6, 8, 16, or 18; and  
(b) a polypeptide characterized by at least 85% sequence identity to the amino acid sequence of (a).

5. The polypeptide according to claim 4, wherein said polypeptide is characterized by pesticidal activity against at least one pest belonging to the order Coleopteran.

6. A pesticidal composition comprising at least one polypeptide according to claim 5 in combination with a carrier.

7. A method for impacting an insect pest comprising applying the pesticidal composition according to claim 6 to the environment of the insect pest by a procedure selected from the group consisting of spraying, dusting, broadcasting, and seed coating.

8. The method according to claim 7, wherein said insect pest is selected from the group consisting of Colorado potato beetle, western corn rootworm, and southern corn rootworm.

9. A transformed plant comprising in its genome at least one stably incorporated nucleotide construct comprising a coding sequence operably linked to a promoter that drives expression of a polypeptide that is pesticidal for at least one pest belonging to the order Coleopteran, wherein said coding sequence is selected from the group consisting of:

- (a) a nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 15, or 17;
- (b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 16, or 18;
- (c) a nucleotide sequence characterized by at least 88% sequence identity to the nucleotide sequence set forth in (a);
- (d) a nucleotide sequence encoding a protein comprising an amino acid sequence characterized by at least 85% sequence identity to the amino acid sequence set forth in (b);
- (e) a nucleotide sequence according to any one of (a) to (d) that comprises codons optimized for expression in a plant;

5 (f) an antisense nucleotide sequence corresponding to the nucleotide sequence of any one of (a) to (d); and

(g) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of any one of (a) to (f).

10. The plant according to claim 9, wherein the plant is a monocot.

11. The plant according to claim 9, wherein said plant is a dicot.

12. Transformed seed of the plant according to claim 9.

13. A transformed microorganism comprising a nucleotide sequence selected from the group consisting of:

15 (a) a nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 15, 17, 27, or 28;

(b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 16, or 18;

(c) a nucleotide sequence characterized by at least 88% sequence identity to the nucleotide sequence set forth in (a);

20 (d) a nucleotide sequence encoding a protein comprising an amino acid sequence characterized by at least 85% sequence identity to the amino acid sequence set forth in (b);

(e) an antisense nucleotide sequence corresponding to the nucleotide sequence of any one of (a) to (d); and

25 (f) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of any one of (a) to (e).

30 14. The transformed microorganism according to claim 13, wherein the nucleotide sequence is operably linked to a promoter that drives expression in said microorganism.

15 A pesticidal composition comprising a transformed microorganism according to claim 13 and a carrier, wherein said composition has been treated to prolong pesticidal activity.

5 16. A method of impacting a pest comprising applying the pesticidal composition according to claim 14 to the environment of the pest by a procedure selected from the group consisting of spraying, dusting, broadcasting, and seed coating.

10 17. A method for impacting a plant pest comprising introducing into said plant or cell thereof at least one nucleotide construct comprising a coding sequence operably linked to a promoter that drives expression of a pesticidal polypeptide in plant cells, wherein said nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence set forth in SEQ ID NO:1, 3, 5, 7, 9, 15, or 17;

15 (b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, 4, 6, 8, 16, or 18;

(c) a nucleotide sequence characterized by at least 88% sequence identity to the nucleotide sequence set forth in (a);

20 (d) a nucleotide sequence encoding a protein comprising an amino acid sequence characterized by at least 85% sequence identity to the amino acid sequence set forth in (b);

(e) an antisense nucleotide sequence corresponding to the nucleotide sequence of any one of (a) to (d); and

25 (f) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of any one of (a) to (e).

18. The method according to claim 17, wherein the plant produces a polypeptide characterized by pesticidal activity against at least one pest of the order Coleoptera.

19. The method according to claim 18, wherein said plant pest is selected from the group consisting of Colorado potato beetle, western corn rootworm, and southern corn rootworm.

5 20. A variant of the nucleic acid set forth in SEQ ID NO:19 wherein the variant comprises a nucleotide sequence having at least one additional codon not present in the nucleotide sequence set forth in SEQ ID NO:19, wherein the at least one additional codon introduces an additional protease-sensitive site in the loop region between alpha-helices 3 and 4 of domain 1 of the encoded polypeptide, and further wherein the  
10 polypeptide encoded by the variant is characterized by improved pesticidal activity against a pest belonging to the order Coleopteran relative to the activity of the polypeptide set forth in SEQ ID NO:2.

15 21. The nucleic acid according to claim 20, wherein the nucleotide sequence is optimized for expression in a plant.

22. An expression cassette comprising a nucleic acid according to claim 20, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a microorganism or in a plant cell.

20 23. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence set forth in any one of SEQ ID NOS: 5, 11, 15, 21, 23, 39, and 43;
- 25 (b) a nucleotide sequence encoding any one of the amino acid sequences set forth in SEQ ID NOS: 6, 12, 16, 22, 24, 40, and 44;
- (c) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the polypeptide comprises an additional protease-sensitive cleavage site inserted between amino acid residues 164 and 165 of SEQ ID NO: 16;
- 30 (d) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the polypeptide variant comprises an additional amino acid sequence designed to

introduce a trypsin cleavage site between amino acid residues 164 and 165 of SEQ ID NO: 16;

(e) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the polypeptide comprises an additional amino acid sequence designed to introduce a chymotrypsin cleavage site between amino acid residues 160 and 161 of SEQ ID NO: 16; and

(f) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the polypeptide comprises an amino acid sequence in which residues corresponding to positions 161 through 163 of SEQ ID NO:16 are removed and additional amino acids comprising a chymotrypsin cleavage site are introduced in their place.

24. The nucleic acid according to claim 23, wherein the nucleotide sequence is optimized for expression in a plant.

25. A variant of the nucleic acid set forth in SEQ ID NO: 15, wherein the variant comprises a nucleotide sequence that includes at least one additional codon that introduces an additional protease-sensitive site in the loop region between alpha-helices 3 and 4 of domain 1 of the polypeptide encoded by the variant nucleic acid, and further wherein the encoded polypeptide is characterized by improved pesticidal activity against a pest belonging to the order Coleopteran relative to the activity of the polypeptide set forth in SEQ ID NO: 2.

26. An expression cassette comprising a nucleic acid according to claim 25, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a microorganism or in a plant cell.

27. An isolated nucleic acid comprising a nucleic acid sequence selected from the group consisting of:

- (a) a nucleotide sequence set forth in SEQ ID NO:19, 29, 31, 33, 41, or 45;
- (b) a nucleotide sequence encoding the amino acid sequence set

forth in SEQ ID NO:20, 30, 32, 34, 42, or 46;

(c) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant encodes a polypeptide characterized by an additional protease-sensitive cleavage site inserted immediately 5' to amino acid 114 of SEQ ID NO: 20;

5 (d) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant encodes a polypeptide characterized by an additional amino acid sequence designed to introduce a trypsin cleavage site between amino acid residues 113 and 114 of SEQ ID NO: 20;

10 (e) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant encodes a polypeptide characterized by an additional amino acid sequence designed to introduce a chymotrypsin cleavage site between amino acid residues 113 and 114 of SEQ ID NO:20; and

15 (f) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant encodes a polypeptide characterized by an amino acid sequence in which the amino acids located at positions 114 through 116 of SEQ ID NO:20 are removed and additional amino acids comprising a chymotrypsin site are inserted in their place.

28. The nucleic acid according to claim 27, wherein the nucleotide sequence is optimized for expression in a plant.

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29. A variant of the polypeptide set forth in SEQ ID NO: 16, wherein the variant comprises an amino acid sequence that includes at least one additional amino acid residue that introduces an additional protease-sensitive site in the loop region between alpha-helices 3 and 4 of domain 1 of the polypeptide, and further wherein the encoded polypeptide is characterized by improved pesticidal activity against a pest belonging to the order Coleopteran relative to the activity of the polypeptide set forth in SEQ ID NO:2.

30. An isolated pesticidal polypeptide comprising an amino acid sequence set forth in SEQ ID NOS:6, 12, 16, 20, 22, 24, 30, 32, 34, 40, 42, 44, or 46.

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31. A transformed plant comprising in its genome at least one stably incorporated nucleotide construct comprising a coding sequence operably linked to a promoter that drives expression of a pesticidal polypeptide in cells of transformed plant, wherein said coding sequence is selected from the group consisting of:

5 (a) a nucleotide sequence set forth in SEQ ID NOS:11, 19, 21, 23, 29, 31, 33, 39, 41, 43, or 45;

(b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:12, 20, 22, 24, 30, 32, 34, 40, 42, 44, or 46;

10 (c) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the variant comprises an additional protease-sensitive cleavage site inserted between amino acid residues 164 and 165 of SEQ ID NO:16;

(d) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the variant comprises an additional amino acid sequence designed to introduce a trypsin cleavage site between amino acid residues 164 and 165 of SEQ ID NO:16;

15 (e) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the variant comprises an additional amino acid sequence designed to introduce a chymotrypsin cleavage site between amino acid residues 160 and 161 of SEQ ID NO: 16;

20 (f) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein amino acid residues corresponding to amino acids 161 through 163 of SEQ ID NO:16 are removed and amino acids comprising a chymotrypsin cleavage site are introduced in their place;

(g) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant encodes a polypeptide characterized by an additional protease-sensitive cleavage site immediately 5' to amino acid 114 of SEQ ID NO: 20;

25 (h) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant encodes a polypeptide characterized by an additional amino acid selected to introduce a trypsin cleavage site immediately 5' of amino acid 114 of SEQ ID NO: 20;

i) a nucleotide sequence comprising a variant of SEQ ID NO:19, wherein the variant comprises additional nucleic acid residues designed to introduce a  
30 chymotrypsin cleavage site between amino acid residues 113 and 114 of SEQ ID NO: 20;



j) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:20, wherein the amino acid residues located at positions 114 through 116 of SEQ ID NO:20 are removed and additional amino acids comprising a chymotrypsin site are in their place; and

5 k) a nucleotide sequence according to any one of (a) to (j) that comprises codons optimized for expression in a plant.

32. The transformed plant according to claim 31, wherein ~~cells of the~~ transformed plant express a polypeptide that is pesticidal for at least one pest belonging to the order Coleoptera.

33. The plant according to claim 32, wherein said plant is a monocot.

34. The plant according to claim 32, wherein said plant is a dicot.

35. Transformed seed of the plant according to claim 32.

36. A transformed microorganism comprising a nucleic acid selected from the group consisting of:

20 (a) a nucleotide sequence set forth in SEQ ID NO:11, 19, 21, 23, 29, 31, 33, 39, 41, 43, or 45;

(b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:12, 20, 22, 24, 30, 32, 34, 40, 42, 44, or 46;

25 (c) a nucleotide sequence encoding a variant of SEQ ID NO:19, wherein the variant comprises a nucleic acid insert designed to introduce an additional protease-sensitive site between amino acid residues 117 and 118 of SEQ ID NO: 20; and

(d) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:16, wherein the variant comprises an additional protease-sensitive site inserted between amino acid residues 164 and 165 of SEQ ID NO:16; and

(e) a nucleotide sequence encoding a polypeptide variant of SEQ ID NO:20, wherein the variant comprises an additional protease-sensitive site inserted between amino acid residues 117 and 118 of SEQ ID NO:20.

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